

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
5 August 2004 (05.08.2004)

PCT

(10) International Publication Number
WO 2004/065623 A3

(51) International Patent Classification⁷: **C12Q 1/26**

(21) International Application Number:
PCT/EP2004/000264

(22) International Filing Date: 15 January 2004 (15.01.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
103 02 370.4 22 January 2003 (22.01.2003) DE

(71) Applicant and

(72) Inventor: **BACHER, Adelbert** [DE/DE]; Königsbergstr.
74, 85748 Garching (DE).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **ADAM, Petra**
[DE/DE]; Engelhofstr. 6, 85386 Dietersheim (DE).
EISENREICH, Wolfgang [DE/DE]; Römerhofweg 51a,

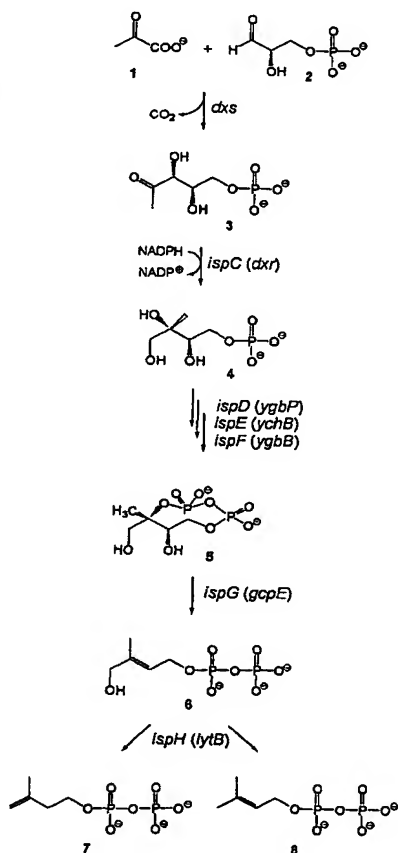
85748 Garching (DE). **GRÄWERT, Tobias** [DE/DE];
Fischergasse 25, 85354 Freising (DE). **HECHT, Ste-
fan** [DE/DE]; Engelhofstr. 6, 85386 Dietersheim (DE).
ROHDICH, Felix [DE/DE]; Moosburger Str. 16, 85406
Zolling (DE). **ZEPECK, Ferdinand** [DE/DE]; Wörthstr.
26, 81667 München (DE).

(74) Agent: **HARTZ, Nikolai, F.**; Wächtershäuser & Hartz,
Weinstr. 8, 80333 München (DE).

(81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,
AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN,
CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI,
GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE,
KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD,
MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG,
PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM,
TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM,
ZW.

[Continued on next page]

(54) Title: ASSAY FOR INHIBITORS OF ISPH



(57) Abstract: Assay for testing a sample for the presence or absence of inhibition of the enzymatic conversion of 1-hydroxy-2-methyl-(E)-butenyl 4-diphosphate into isopentenyl diphosphate and/or dimethylallyl diphosphate by the following steps: a) reacting an aqueous mixture containing 1-hydroxy-2-methyl-(E)-butenyl 4-diphosphate, a 1-hydroxy-2-methyl-(E)-butenyl 4-diphosphate reductase, and a reducing agent under predetermined reaction conditions for a predetermined period of time; b) analyzing the reaction mixture obtained in step (a) for the consumed amount of 1-hydroxy-2-methyl-(E)-butenyl 4-diphosphate and/or NAD(P)H and/or for the produced amount of isopentenyl diphosphate, and/or dimethylallyl diphosphate and/or NAD(P)⁺; c) repeating step (a) in the presence of the sample to be tested; d) repeating step (b) with the reaction mixture defined in step (c); e) comparing the results of steps (b) and (d).



(84) **Designated States** (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

(88) **Date of publication of the international search report:**
30 September 2004

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP2004/000264

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12Q1/26

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, EMBASE, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 02/083720 A (EISENREICH WOLFGANG ; ROHDICH FELIX (DE); ADAM PETRA (DE); BACHER ADEL) 24 October 2002 (2002-10-24) cited in the application page 13, last paragraph - page 14, paragraph 1 page 18, line 15 - line 23 page 20, line 15 - page 21, line 4 examples 33,45 claims 58,64,67,71	1,7,8,10
Y	----- -/-	1,7,8,10

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

26 July 2004

Date of mailing of the international search report

06/08/2004

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Weber, P

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP2004/000264

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>ALTINCICEK B ET AL: "LytB protein catalyzes the terminal step of the 2-C-methyl-D-erythritol-4-phosphate pathway of isoprenoid biosynthesis" FEBS LETTERS, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 532, no. 3, 18 December 2002 (2002-12-18), pages 437-440, XP004398446 ISSN: 0014-5793 page 438, left-hand column, paragraph 2.3 - paragraph 2.4 page 439, right-hand column, line 3 - line 17 page 440, left-hand column, line 17 - line 23</p>	1,9,10
Y	<p>ADAM PETRA ET AL: "Biosynthesis of terpenes: studies on 1-hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate reductase." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA. 17 SEP 2002, vol. 99, no. 19, 17 September 2002 (2002-09-17), pages 12108-12113, XP002289308 ISSN: 0027-8424 cited in the application page 12109, left-hand column, last paragraph - right-hand column, paragraph 1 page 12109, right-hand column, last paragraph - page 12110, left-hand column, paragraph 1 page 12111, right-hand column, line 15 - line 20 page 12112, left-hand column, line 15 - right-hand column, line 2</p>	1,7,8,10
A	<p>OLLAGNIER S ET AL: "Activation of the anaerobic ribonucleotide reductase from Escherichia coli: The essential role of the iron-sulfur center for S-adenosylmethionine reduction" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 272, no. 39, 1997, pages 24216-24223, XP002289404 ISSN: 0021-9258 abstract page 24222, left-hand column, line 4 - line 8</p> <p style="text-align: center;">----- -/-</p>	2

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP2004/000264

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	<p>WO 2004/035810 A (SANDERBRAND SILKE ; BIOAGENCY AG (DE); HINTZ MARTIN (DE); JOMAA HASSAN) 29 April 2004 (2004-04-29) page 6, line 1 - line 3 example 6 claims 1,4-7,12,23,24</p>	1,9,10

INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP2004/000264

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of Item 1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:
 - a. type of material
 - ☒ a sequence listing
 - ☐ table(s) related to the sequence listing
 - b. format of material
 - ☒ in written format
 - ☒ in computer readable form
 - c. time of filing/furnishing
 - ☐ contained in the international application as filed
 - ☐ filed together with the international application in computer readable form
 - ☒ furnished subsequently to this Authority for the purpose of search
2. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 2004/000264

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.: 11
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.2

Claims Nos.: 11

Present claim 11 relates to a compound defined by reference to a desirable characteristic or property, namely its identification of being an inhibitor via one of several claimed assays. The claim covers all compounds having this characteristic or property, whereas the application provides no support within the meaning of Article 6 PCT and no disclosure within the meaning of Article 5 PCT for such compounds. In the present case, the claim so lacks support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claim also lacks clarity (Article 6 PCT). An attempt is made to define the compound by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, no search has been carried out for the claim.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.5), should the problems which led to the Article 17(2) declaration be overcome.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP2004/000264

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 02083720	A	24-10-2002	DE 10201458 A1	17-10-2002
			CA 2443874 A1	24-10-2002
			WO 02083720 A2	24-10-2002
			EP 1377663 A2	07-01-2004
WO 2004035810	A	29-04-2004	DE 10247478 A1	24-06-2004
			WO 2004035810 A2	29-04-2004